

IAP12 Rec'd FCT/PTO 27 APR 2006

## SEQUENCE LISTING

&lt;110&gt; KANEKA CORPORATION

&lt;120&gt; NOVEL ACETOACETYL-CoA REDUCTASE AND PROCESS FOR PRODUCING OPTICALLY ACTIVE ALCOHOL

&lt;130&gt; BO30435WO01

&lt;150&gt; JP2003-380987

&lt;151&gt; 2003-11-11

&lt;160&gt; 15

&lt;170&gt; PatentIn version 3.1

&lt;210&gt; 1

&lt;211&gt; 245

&lt;212&gt; PRT

&lt;213&gt; Achromobacter xylosoxidans subsp. denitrificans

&lt;400&gt; 1

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Ser | Gly | Lys | Leu | Ala | Tyr | Val | Thr | Gly | Gly | Met | Gly | Gly | Ile | Gly |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Thr | Ser | Ile | Cys | Gln | Arg | Leu | Ala | Lys | Asp | Gly | Phe | Arg | Val | Val | Ala |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gly | Cys | Gly | Pro | Ser | Arg | Asn | Tyr | Gln | Gln | Trp | Leu | Asp | Glu | Gln | Ala |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Gln | Gly | Tyr | Thr | Phe | Tyr | Ala | Ser | Val | Gly | Asn | Val | Ser | Asp | Trp |
|     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Ser | Thr | Val | Glu | Ala | Phe | Glu | Arg | Val | Lys | Arg | Asp | Met | Gly | Pro |
| 65  |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Val | Asp | Val | Leu | Val | Asn | Asn | Ala | Gly | Ile | Thr | Arg | Asp | Gly | Leu | Phe |
|     |     |     | 85  |     |     |     |     |     | 90  |     |     |     |     | 95  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Lys | Met | Ser | Ala | Asp | Asp | Trp | Arg | Ala | Val | Ile | Asp | Thr | Asn | Leu |
|     |     |     | 100 |     |     |     |     | 105 |     |     |     |     |     | 110 |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asn | Ser | Leu | Phe | Asn | Val | Thr | Lys | Gln | Val | Ile | Asp | Asp | Met | Val | Glu |
|     |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Gln | Trp | Gly | Arg | Ile | Val | Asn | Ile | Ser | Ser | Val | Asn | Gly | Gln | Lys |
|     | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gly | Gln | Phe | Gly | Gln | Thr | Asn | Tyr | Ser | Thr | Ala | Lys | Ala | Gly | Ile | His |
| 145 |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gly | Phe | Thr | Met | Ala | Leu | Ala | Gln | Glu | Val | Ala | Ser | Lys | Gly | Ile | Thr |
|     |     |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Val | Asn | Thr | Val | Ser | Pro | Gly | Tyr | Ile | Gly | Thr | Asp | Met | Val | Arg | Ala |
|     |     |     | 180 |     |     |     |     | 185 |     |     |     |     |     | 190 |     |

Ile Arg Pro Asp Val Leu Glu Lys Ile Val Ala Thr Ile Pro Val Arg  
195 200 205

Arg Leu Gly Thr Pro Glu Glu Ile Ala Ser Ile Thr Ser Trp Leu Ala  
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Ser Asp Glu Ser Gly Phe Ser Thr Gly Ala Asp Phe Ser Leu Asn Gly  
225 230 235 240

Gly Leu His Met Gly  
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| Met Ser Gly Lys Leu Ala Tyr Val Thr Gly Gly Met Gly Gly Ile Gly |     |
| 1 5 10 15   |     |
| acc tca att tgc cag cgc ctg gcc aaa gat ggc ttt cgc gtg gtg gca | 96  |
| Thr Ser Ile Cys Gln Arg Leu Ala Lys Asp Gly Phe Arg Val Val Ala |     |
| 20 25 30  |     |
| ggc tgc ggc ccc agc cgc aat tac cag caa tgg ctg gat gaa cag gcg | 144 |
| Gly Cys Gly Pro Ser Arg Asn Tyr Gln Gln Trp Leu Asp Glu Gln Ala |     |
| 35 40 45  |     |
| gcg cag ggc tat acg ttc tac gcg tca gtg ggc aac gtg tcc gat tgg | 192 |
| Ala Gln Gly Tyr Thr Phe Tyr Ala Ser Val Gly Asn Val Ser Asp Trp |     |
| 50 55 60  |     |
| gag tcc acg gta gaa gca ttc gag cgc gtc aag cgg gac atg ggc ccg | 240 |
| Glu Ser Thr Val Glu Ala Phe Glu Arg Val Lys Arg Asp Met Gly Pro |     |
| 65 70 75 80   |     |
| gtc gat gtg ctg gtc aac aac gcg ggc atc acc cgc gac ggc ctg ttc | 288 |
| Val Asp Val Leu Val Asn Asn Ala Gly Ile Thr Arg Asp Gly Leu Phe |     |
| 85 90 95  |     |
| cgc aag atg agc gcc gac gac tgg cgc gcg gtc atc gac acc aac ctg | 336 |
| Arg Lys Met Ser Ala Asp Asp Trp Arg Ala Val Ile Asp Thr Asn Leu |     |
| 100 105 110   |     |
| aac agc ctc ttc aac gtg acc aag cag gtg atc gac gac atg gtc gag | 384 |
| Asn Ser Leu Phe Asn Val Thr Lys Gln Val Ile Asp Asp Met Val Glu |     |
| 115 120 125   |     |
| cgc cag tgg ggc cgc atc gtc aac atc agc tcg gtg aac ggg cag aag | 432 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
| Arg | Gln | Trp | Gly | Arg | Ile | Val | Asn | Ile | Ser | Ser | Val | Asn | Gly | Gln | Lys |     |  |
| 130 |     |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |     |  |
| ggg | cag | ttc | ggc | cag | acg | aac | tat | tcc | acg | gcg | aag | gcg | ggc | atc | cat | 480 |  |
| Gly | Gln | Phe | Gly | Gln | Thr | Asn | Tyr | Ser | Thr | Ala | Lys | Ala | Gly | Ile | His |     |  |
| 145 |     |     |     |     | 150 |     |     |     | 155 |     |     |     |     |     | 160 |     |  |
| ggc | ttc | acc | atg | gcg | ctg | gcg | cag | gaa | gtg | gcc | agc | aag | ggc | atc | acg | 528 |  |
| Gly | Phe | Thr | Met | Ala | Leu | Ala | Gln | Glu | Val | Ala | Ser | Lys | Gly | Ile | Thr |     |  |
|     |     |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |     |  |
| gtc | aac | acg | gtg | tcg | ccg | ggc | tac | atc | ggc | acg | gac | atg | gtt | cgc | gcc | 576 |  |
| Val | Asn | Thr | Val | Ser | Pro | Gly | Tyr | Ile | Gly | Thr | Asp | Met | Val | Arg | Ala |     |  |
|     |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |     |  |
| atc | cgt | ccg | gac | gtg | ctg | gaa | aag | atc | gtc | gcc | acc | att | ccg | gtg | cgc | 624 |  |
| Ile | Arg | Pro | Asp | Val | Leu | Glu | Lys | Ile | Val | Ala | Thr | Ile | Pro | Val | Arg |     |  |
|     |     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |     |  |
| cgc | ctg | ggc | acg | ccg | gag | gaa | atc | gcg | tcc | atc | acg | tcg | tgg | ctg | gcc | 672 |  |
| Arg | Leu | Gly | Thr | Pro | Glu | Glu | Ile | Ala | Ser | Ile | Thr | Ser | Trp | Leu | Ala |     |  |
|     | 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |     |  |
| tcg | gat | gag | tct | ggg | ttt | tcg | acg | ggc | gcg | gac | ttc | tcg | ctc | aac | ggc | 720 |  |
| Ser | Asp | Glu | Ser | Gly | Phe | Ser | Thr | Gly | Ala | Asp | Phe | Ser | Leu | Asn | Gly |     |  |
| 225 |     |     |     |     | 230 |     |     |     | 235 |     |     |     |     |     | 240 |     |  |
| ggc | ctg | cat | atg | ggc | tga |     |     |     |     |     |     |     |     |     |     | 738 |  |
| Gly | Leu | His | Met | Gly |     |     |     |     |     |     |     |     |     |     |     |     |  |
|     |     |     |     | 245 |     |     |     |     |     |     |     |     |     |     |     |     |  |

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 Gly Cys Gly Pro Asn Ser Pro Arg Arg Glu Lys Trp Leu Glu Gln Gln  
 35 40 45  
 Lys Ala Leu Gly Phe Asp Phe Ile Ala Ser Glu Gly Asn Val Ala Asp  
 50 55 60  
 Trp Asp Ser Thr Lys Thr Ala Phe Asp Lys Val Lys Ser Glu Val Gly  
 65 70 75 80  
 Glu Val Asp Val Leu Ile Asn Asn Ala Gly Ile Thr Arg Asp Val Val  
 85 90 95  
 Phe Arg Lys Met Thr Arg Ala Asp Trp Asp Ala Val Ile Asp Thr Asn

| 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Thr | Ser | Leu | Phe | Asn | Val | Thr | Lys | Gln | Val | Ile | Asp | Gly | Met | Ala |
|     |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |
| Asp | Arg | Gly | Trp | Gly | Arg | Ile | Val | Asn | Ile | Ser | Ser | Val | Asn | Gly | Gln |
|     | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |
| Lys | Gly | Gln | Phe | Gly | Gln | Thr | Asn | Tyr | Ser | Thr | Ala | Lys | Ala | Gly | Leu |
| 145 |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |
| His | Gly | Phe | Thr | Met | Ala | Leu | Ala | Gln | Glu | Val | Ala | Thr | Lys | Gly | Val |
|     |     |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |
| Thr | Val | Asn | Thr | Val | Ser | Pro | Gly | Tyr | Ile | Ala | Thr | Asp | Met | Val | Lys |
|     |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |
| Ala | Ile | Arg | Gln | Asp | Val | Leu | Asp | Lys | Ile | Val | Ala | Thr | Ile | Pro | Val |
|     |     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |
| Lys | Arg | Leu | Gly | Leu | Pro | Glu | Glu | Ile | Ala | Ser | Ile | Cys | Ala | Trp | Leu |
|     | 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |
| Ser | Ser | Glu | Glu | Ser | Gly | Phe | Ser | Thr | Gly | Ala | Asp | Phe | Ser | Leu | Asn |
| 225 |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |
| Gly | Gly | Leu | His | Met | Gly |     |     |     |     |     |     |     |     |     |     |
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| Met Thr Gln Arg Ile Ala Tyr Val Thr Gly Gly Met Gly Gly Ile Gly |     |
| 1 5 10 15   |     |
| acc gcc att tgc cag cgg ctg gcc aag gat ggc ttt cgt gtg gtg gcc | 96  |
| Thr Ala Ile Cys Gln Arg Leu Ala Lys Asp Gly Phe Arg Val Val Ala |     |
| 20 25 30  |     |
| ggt tgc ggc ccc aac tcg ccg cgc cgc gaa aag tgg ctg gag cag cag | 144 |
| Gly Cys Gly Pro Asn Ser Pro Arg Arg Glu Lys Trp Leu Glu Gln Gln |     |
| 35 40 45  |     |
| aag gcc ctg ggc ttc gat ttc att gcc tcg gaa ggc aat gtg gct gac | 192 |
| Lys Ala Leu Gly Phe Asp Phe Ile Ala Ser Glu Gly Asn Val Ala Asp |     |
| 50 55 60  |     |
| tgg gac tcg acc aag acc gca ttc gac aag gtc aag tcc gag gtc ggc | 240 |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|-----|
| Trp | Asp | Ser | Thr | Lys | Thr | Ala | Phe | Asp | Lys | Val | Lys | Ser | Glu | Val | Gly |  |     |
| 65  |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     |     | 80  |  |     |
| gag | gtt | gat | gtg | ctg | atc | aac | aac | gcc | ggg | atc | acc | cgc | gac | gtg | gtg |  | 288 |
| Glu | Val | Asp | Val | Leu | Ile | Asn | Asn | Ala | Gly | Ile | Thr | Arg | Asp | Val | Val |  |     |
|     |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |  |     |
| ttc | cgc | aag | atg | acc | cgc | gcc | gac | tgg | gat | gcg | gtg | atc | gac | acc | aac |  | 336 |
| Phe | Arg | Lys | Met | Thr | Arg | Ala | Asp | Trp | Asp | Ala | Val | Ile | Asp | Thr | Asn |  |     |
|     |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |  |     |
| ctg | acc | tcg | ctg | ttc | aac | gtc | acc | aag | cag | gtg | atc | gac | ggc | atg | gcc |  | 384 |
| Leu | Thr | Ser | Leu | Phe | Asn | Val | Thr | Lys | Gln | Val | Ile | Asp | Gly | Met | Ala |  |     |
|     |     |     | 115 |     |     |     | 120 |     |     |     |     | 125 |     |     |     |  |     |
| gac | cgt | ggc | tgg | ggc | cgc | atc | gtc | aac | atc | tcg | tcg | gtg | aac | ggg | cag |  | 432 |
| Asp | Arg | Gly | Trp | Gly | Arg | Ile | Val | Asn | Ile | Ser | Ser | Val | Asn | Gly | Gln |  |     |
|     | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |  |     |
| aag | ggc | cag | ttc | ggc | cag | acc | aac | tac | tcc | acc | gcc | aag | gcc | ggc | ctg |  | 480 |
| Lys | Gly | Gln | Phe | Gly | Gln | Thr | Asn | Tyr | Ser | Thr | Ala | Lys | Ala | Gly | Leu |  |     |
|     | 145 |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |  |     |
| cat | ggc | ttc | acc | atg | gca | ctg | gcg | cag | gaa | gtg | gcg | acc | aag | ggc | gtg |  | 528 |
| His | Gly | Phe | Thr | Met | Ala | Leu | Ala | Gln | Glu | Val | Ala | Thr | Lys | Gly | Val |  |     |
|     |     |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |  |     |
| acc | gtc | aac | acg | gtc | tct | ccg | ggc | tat | atc | gcc | acc | gac | atg | gtc | aag |  | 576 |
| Thr | Val | Asn | Thr | Val | Ser | Pro | Gly | Tyr | Ile | Ala | Thr | Asp | Met | Val | Lys |  |     |
|     |     |     |     | 180 |     |     |     | 185 |     |     |     |     | 190 |     |     |  |     |
| gcg | atc | cgc | cag | gac | gtg | ctc | gac | aag | atc | gtc | gcg | acg | atc | ccg | gtc |  | 624 |
| Ala | Ile | Arg | Gln | Asp | Val | Leu | Asp | Lys | Ile | Val | Ala | Thr | Ile | Pro | Val |  |     |
|     |     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |  |     |
| aag | cgc | ctg | ggc | ctg | ccg | gaa | gag | atc | gcc | tcg | atc | tgc | gcc | tgg | ttg |  | 672 |
| Lys | Arg | Leu | Gly | Leu | Pro | Glu | Glu | Ile | Ala | Ser | Ile | Cys | Ala | Trp | Leu |  |     |
|     | 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |  |     |
| tcg | tcg | gag | gag | tcc | ggg | ttc | tcg | acc | ggc | gcc | gac | ttc | tcg | ctc | aac |  | 720 |
| Ser | Ser | Glu | Glu | Ser | Gly | Phe | Ser | Thr | Gly | Ala | Asp | Phe | Ser | Leu | Asn |  |     |
|     | 225 |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |  |     |
| ggc | ggc | ctg | cat | atg | ggc | tga |     |     |     |     |     |     |     |     |     |  | 741 |
| Gly | Gly | Leu | His | Met | Gly |     |     |     |     |     |     |     |     |     |     |  |     |
|     |     |     |     | 245 |     |     |     |     |     |     |     |     |     |     |     |  |     |

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19

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25

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ctttcgcggtgtgggcaggctgcggccccagccgcaattacagcaatggctggatgaaca 180  
ggcggcgcgaggctatacgttctacgcgtcagtgggcaacgtgtccgattgggagtccac 240  
ggtagaagcatcagcgctcaagcgggacatggggcccggtcgatgtgttggtcaacaa 300  
cgcggggcacacccgcgacggcctgttccgcaagatgagcgccgacgactggcgcgcgggt 360  
catcgacacc aacctgaacgcctcttcaa cgtgaccaagcaggtgatcgacgacatgggt 420  
cgagcgccagtggggccgcatcgtcaacatcagctcggtagacgggcagaggggcagtt 480  
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